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Karyotypes of some Indian Noctuid Moths (Lepidoptera)

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Abstract. Chromosome study during meiosis in eleven species of Indian noctuid moths (*Achaea janata, Acontia intersepta, Anomis sabulifera, Cosmophila erosa, Earias fabia, Heliothis armigera, Hyblaea puera, Plusia orichalcea, Plusia signata, Sesamia inferens, Tarache tropica*) established the haploid number as 31 in all the species. The structure and behaviour of the chromosomes during the meiotic cycle and the possible chromosome evolution in this family have been examined.

Introduction

The latest review on Lepidopteran chromosomes (c.f. Robinson, 1971) includes spcies belonging to family Noctuidae. Karyotypes of additional species have been determined in recent years including some Indian species (Rishi, 1973, 1975; Nayak, 1975). The present paper incorporates additional information on the cytologenetics of eleven species of Indian Noctuid moths.

Material & Methods

All the materials were collected in close vicinity of Bhubaneswar and were identified by Z. S. I. staff of Calcutta. For ease of reference, the species along with their host plants, have been listed in Table 1. The males were found to be most active meiotically in the 3rd to 5th instars. The larval testes were dissected out in hypotonic solution (0.45% sodium citrate solution) and fixed overnight in 1:3 acetic alcohol. Permanent squash and smear preparations of the material were made and the slides were stained in Heidenhains' iron-alum haematoxylin. Slides were examined under a Meopta Binocular Research Microscope and good metaphase stages were scored using 100X oil immersion objective and 15X ocular with the help of camera lucida. Some of the stages were photomicrographed.

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Table 1

Sl. Number	Family (Noctuidae) & Species	Foodplant
1	*Achaea janata* Linn.	*Ricinus communis*
2	*Acontia intersepta* Guen.	*Hibiscus esculentus*
3	*Anomis sabulifera* Guen. (Jute looper)	*Choreorus* sp.
4	*Cosmophila erosa* Hubn. (Green semi-looping larva)	*Hibiscus esculentus*
5	*Earias fabia* Stoll.	*Hibiscus esculentus*
6	*Heliothis armigera* Hubn.	*Cicer arietinum*
7	*Hyblaea puera* Cram.	*Tecona grandis*
8	*Plusia orichalcea* Fabr.	*Brassica oleracea*
9	*Plusia signata* Fabr.	*Nicotiana* sp.
10	*Sesamia inferens* Walk. (Pink borer)	*Eleusine coracana*
11	*Tarache tropica* Guen.	Unidentified

Observations

Achaea janata

2n = 62. Early meiotic prophase chromosomes, especially the diplotene and diakinetic bivalents were of particular morphological interest showing a fuzzy contour, similar to Lamp-brush chromosomes. However, at late diakinesis and metaphase stages, these fringes disappeared. Metaphase I cells usually showed 31 bivalents, but very often contained several univalents. This situation even occurred in many diakinetic cells in which some of the separated elements showed chromatinic interconnection between pairs and they did not resolve completely. This further shows that the unpaired chromosomes had undergone normal segregation and were the homologues of bivalents which formed no chiasma or weak chiasma. Metaphase II showed 31 univalents (Figs. 1-3).

Acontia intersepta

2n = 62. Metaphase I cells showed 31 bivalents. In some of these cells, precocious resolution of a bivalent into univalents was marked. At anaphase I, which is very short-lived, separation of homologues was synchronous, although in a good number of plates partners of a bivalent still lagged behind on the equator when all others had almost reached the poles. This may be presumed as the XX-sex chromosome pair, making inferences drawn from other sources. Metaphase II showed 31 univalents indicating the regular segragation of unpaired elements of metaphase I (Figs. 4-6).

Anomis sabulifera

2n = 62. Metaphase I cells showed 31 bivalents. Precocious separation of a bivalent into two univalents was noticed in a number of pro-metaphase and metaphase I cells. Both in early and mid-anaphase I, two separating elements of one bivalent, presumed as the sex-bivalent, still remained on the equatorial plate when all other bivalents had their homologues moving towards the poles. Even in some late anaphase and early telophase stages the two separated elements were seen to be present on the bridge of the spindle fibres joining two daughter nuclei under formation. If this pair is the same that as with lagging anaphasic movement, then it could be equally argued to represent the products of 'elimination chromatin' to be ultimately lost. Metaphase II showed 31 univalents confirming the haploid number as n = 31 (Figs. 7-9).

Cosmophila erosa

2n = 62. Metaphase I cells showed 31 bivalents but cells with 30 bivalents were also observed. In some cells, one bivalent had been resolved into two univalents (indicated by their small size) resulting in 32 elements. In two late anaphase I cells, separating elements of a bivalent exhibited the characteristic lagging behaviour. Metaphase II plates showed 31 univalents (Figs. 10-12).

Earias fabia

2n = 62. In some of the spermatogonial cells, two of the chromosomes (sex-chromosomes?) were more deeply stained. Metaphase I cells showed 31 bivalents, one of which was more deeply stained than the rest. In some of the cells, in equatorial view, 32 elements were observed where one of the bivalents had undergone early resolution into small-sized homologues. Intermediate forms were also noticed where components of this bivalent did not part but remained connected to one other at one point. In the ensuing anaphase, separation of the sister chromosomes were quite synchronous in every bivalent. Metaphase II plates showed 31 small univalents (Figs. 13-15).

Heliothis armigera

2n = 62. Metaphase I cells showed 31 bivalents. One of the bivalents had undergone early resolution and such resolution was also noticed even in some pre-metaphase plates. Anaphase I was normal but certain dividing cells exhibited two late separating elements (may be the homologues of a bivalent) on the equatorial region. Metaphase II showed 31 univalents (Figs. 16-18).

Hyblaea puera

2n = 62. Two of the elements, probably sex-chromosomes, were differentiated either by heteropycnotic behaviour or by their association

with each other by chromatinic interconnection. Metaphase I cells showed 31 bivalents, out of which one bivalent (presumed to be the sex-bivalent) was quite often more deeply stained. In one early metaphase I cell, one bivalent was ring-like due to delayed condensation. In several metaphase I cells, early resolution of a bivalent into its homologues was noticed. Metaphase II cells showed 31 univalents (Figs. 19-21).

Plusia orichalcea

2n = 62. The number of metaphase I bivalents was 31. One of the bivalents (sex-bivalent?) appeared to be more intensely stained. In some cells an early separation of a bivalent into homologues was encountered. In anaphase I all the bivalents separated synchronously, although occasionally, two separating elements of a bivalent, probably the sex-bivalent, still remained together on the equatorial plate while the remainder moved towards the poles. In many late anaphase-I cells an irregular and clumped chromatinic mass was found in between the separating chromosomes. This clumped mass might be the 'elimination chromatin' although its occurrence has been reported early in the females. Metaphase II cells showed 31 univalents (Figs. 22-24).

Plusia signata

2n = 62. Metaphase I cells showed 31 bivalents, out of which early separation of a single bivalent was seen in many cells while resolution of a number of bivalents into univalents was marked in others. Occasionally, in addition to bivalents and univalents a number of "ringforms", perhaps due to chromatinic interconnections between the ends of separating homologues of bivalents, were observed. Again, rarely, almost all bivalents were found to have resolved into their homologues before onset of anaphase. In anaphase I, separation of homologues was more or less synchronous although, in a number of anaphase cells, one bivalent or the resolved elements of it, showed lagging movement and remained in between the separating groups of chromosomes. Metaphase II cells showed 31 univalents (Figs. 25-27).

Sesamia inferens

2n = 62. Metaphase I cells showed 31 bivalents. Certain cells, however, contained more than 31 chromosomes, possibly due to early resolution of some of the bivalents. A number of tetraploid cells with double the number of bivalents of normal spermatocytes were also recorded. In almost all the anaphase I cells, the bivalents separated into two equal halves simultaneously, although in certain cells one bivalent (or two separating elements probably homologues of the sex-bivalent) still lingered on the equatorial region when the remaining separated chromosomes nearly reached their respective poles. Late anaphase I chromosomes resolved into their chromatids as they reached the poles. Metaphase II cells showed

31 univalents (Figs. 28-30).

Tarache tropica

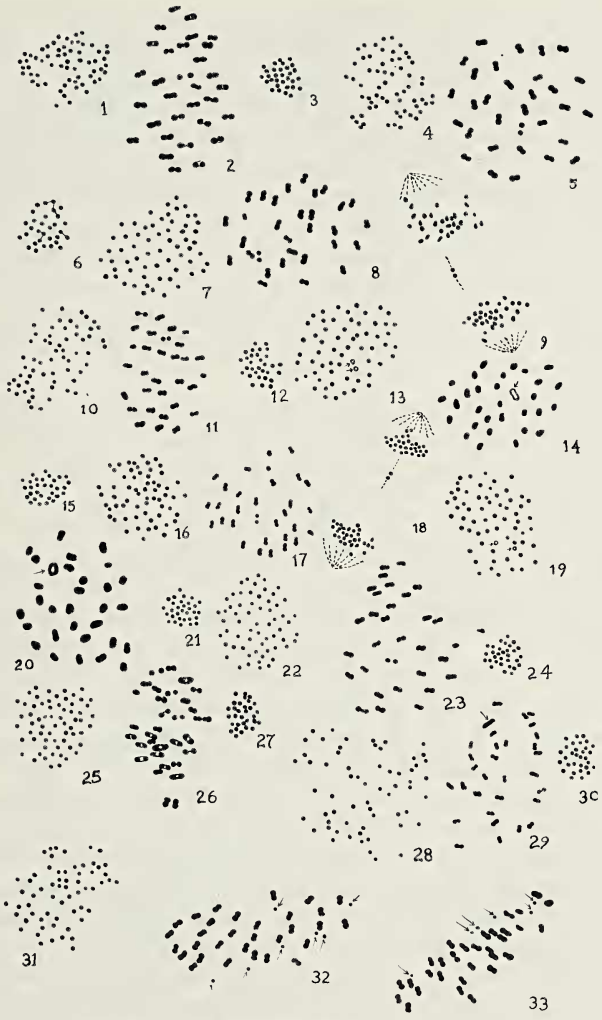
2n = 62. In addition to 31 bivalents, one pre-metaphase cell contained 3 minute dot-like chromosomes which were very likely of supernumeraries. Besides the normal 31 bivalents, metaphase I cells showed a variable number (3 to 7) of minute chromosomal elements. These minute elements, could be either true supernumerary chromosomes or the non-homologous unpaired chromosomes of interspecific hybrids, the latter not uncommon in nature. Distinct metaphase II cells were rare (Figs. 31-33).

Discussion

White (1973) emphasized the need for studying the chromosomes of insects since cytogenetics has contributed to insect systematics in several ways as species which are not always morphologically differentiated can be ascertained as distinct species when difference is noted in their karyotype. Very little attention has been paid to the study of the cytology of Lepidoptera, although much work in this group has been carried out on ecology and control of pests. To be specific, information on the chromosome numbers of Lepidoptera is available for about only one percent of the total number of species of this order. In India, studies on this aspect are negligible. The reason the study of Lepidoptera chromosomes is neglected include:

1. The chromosomes are extremely small, oval, dot-like or almost spherical, or isodiametric and compact bodies yielding no information regarding the position of the centromere and other morphological details.
2. Chromosomes show a marked tendency of clumping together.
3. Ill-defined meiotic stages.
4. Anomalous nature of centromere and sex-chromosomes.

That the Lepidopteran chromosomes, except for minor differences in size, present remarkable uniformity in morphology and behaviour during the meiotic cycle have been well documented (Seiler, 1914; Beliajeff, 1930; Federly, 1938; Lorkovic, 1941; Gupta, 1964; Suomalainen, 1969; Rishi, 1973, 1975; Nayak, 1975; Ennis, 1976). The present investigations confirm earlier findings in that the chromosomes are homomorphic, minute-sized elements presenting a circular disposition both in mitotic and meiotic metaphase stages. A high chromosome number (2n = 62) found in the species investigated is not uncommon for the haploid chromosome number in Lepidoptera extends between 7 in *Erebia aethiopellus* (de Lesse, 1959b) to n = 223 in *Lysandra atlantica* (de Lesse, 1970). In majority species of the group the haploid number falls between 29 to 31. Beliajeff (1930) considers 30 as the ancestral number both for Lepidoptera and Trichoptera which have many features in common including a diffuse centromeric activity, female heterogamety, lack of visible chismata



Figs.1-3. Chromosomes of *Achaea janata.*
Figs. 4-6. Chromosomes of *Acontia intersepta.*
Figs. 7-9. Chromosomes of *Anomis sabulifera,*
Figs. 10-12. Chromosomes of *Cosmophila erosa.*
Figs. 13-15. Chromosomes of *Earias fabia.*
Figs. 16-18. Chromosomes of *Heliothis armigera.*
Figs. 19-21. Chromosomes of *Hyblaea puera.*
Figs. 22-24. Chromosomes of *Plusia orichalcea.*
Figs. 25-27. Chromosomes of *Plusia signata.*
Figs. 28-30. Chromosomes of *Sesamia inferens.*
Figs. 31-33. Chromosomes of *Tarache tropica.*

in the female, occurrence of elimination chromatin in the first meiotic division of the egg, the formation of apyrene sperms and the occurrence of close modal haploid number. Virkki (1963) considers 60 to be modal diploid number. On the other hand White (1954, p. 176) considered 31 as the most frequent haploid number in the members of the group studied to that date with 29, 30 and 31 so nearly equally frequent that he did not feel any of these numbers to be considered as the type number in preference to the others. In the present investigations, chromosome numbers of all the eleven species of the noctuids have a haploid chromosome number n = 31 which agrees with the modal haploid chromosome number (n = 31) established for this family and for Lepidoptera in general (Saitoh, 1959; Bigger, 1960, 1961; Gupta, 1964; Robinson, 1971; Saitoh *et. al.*, 1971; Nayak, 1975).

Very few species of Lepidoptera have been reported so far to have chromosomes as supernumerary elements in their karyotype (Maeki & Makino, 1953; de Lesse, 1960, 1967; Maeki & Ae, 1966; de Lesse & Brown, 1971; Bigger, 1976; Rao & Murty, 1976; Nayak, 1978; Padhi & Nayak, 1981(82)). It is, of course, difficult to distinguish genuine supernumerary chromosomes in the Lepidoptern karyotype which might include a large number of highly contracted chromosomes or even early resolved minute univalents. In the present report, *Tarache tropica* shows numerical variation of 3 to 7 supernumeraries in its karyotype. The number varies from cell to cell and from follicle to follicle. Robinson (1971) considers supernumerary elements as small segments of normal chromosomes produced by accidental breakage. In Lepidoptera, since fragmentation is a common aspect of chromosome evolution, the origin of supernumeraries, as held by him, no doubt, is a special case since the produce of such accidental breakage is likely to behave as a normal chromosome of the regular karyotype in the absence of any localised centromere. Bigger (1976) reported the presence of ß-chromosomes in British material of both *Pieris rapae* and *Pieris napi* and is of the opinion that the supernumeraries are true additional chromosomes and not small fragments of the normal karyotype. Although the exact mode of supernumerary origin is debatable we are of the same opinion as that of White (1973) who considers them to have arisen through fragmentation of heterochromatic blocks of normal chromosomes and to have no evolutionary role in the increase of chromosome number.

Information on the sex-chromosomes of Lepidoptera is meagre since in most species they are not distinguishable from the autosomes. However, there is a female heterogamety with XO or XY females and XX males. Seiler (1958) reports both XO and XY females in *Solenobia triquetrella*. A heteropycnotic pair of chromosome associated with the nucleolus during the spermatogenesis in *Philosamia cynthia* was considered as the sex-chromosome pair by Kawaguchi (1937). Kurihara (1929) had a similar

opinion in *Bombyx mori*. Others considered the deeply stained feulgen positive body sometimes associated with the nucleolus during the indescrete diplotene, as well as the autosomal elements clumped together due to non-specific stickiness, as the sex-chromosome pair (Federley, 1913; Seiler, 1914; Kernewitz, 1915; Gupta, 1964). A pair of chromosomes larger than others frequently occurring in species with high chromosome numbers have been considered as the sex-chromosomes by many workers (Bauer, 1943; White, 1954, 1973; Suomalainen, 1969, 1971). Ennis (1976), investigating chromosome numbers of Canadian Lepidoptera, observed the frequent occurrence of a similar larger pair in a number of lower chromosome numbered taxa, suggesting that in many species the larger pair merely represent an autosomal fusion product. Bigger (1975) found in his observations in *Polyommatus icarus* and *Pieris brassicae* that the largest chromosome is only marginally larger than the other large chromosomes compared with those of other butterflies. Thus size alone cannot be the parameter to distinguish the sex-bivalent. Traut and Mosbacher (1968) distinguished sex-chromatin as a distinctly heteropycnotic body in the somatic interphase nuclei in the females of 70 out of 83 species examined and took it as the Y-chromosome, since the presence of such body was not observed in the corresponding males. Traut and Rathjens (1973) used the fluorescent Feulgen technique to demonstrate the W (= Y) chromosome in the early oocyte and nurse cells. Suomalainen (1969) demonstrated the occurrence of distinct sex-chromatin bodies in somatic tissues of females of *Witlesia crataegella, Scoparia ambigualis* and *Bactra robustana* and suggested this to be the heteropycnotic Y-chromosome. Bigger (1975), by employing G-banding technique, furnished strong evidence in support of the sex-chromatin body as the X-chromosome in *Pieris brassicae* and *Polyommatus icarus* where in a small proportion of interphases he clearly observed a heteropycnotic body which corresponded very closely to the structure of G-banded X-chromosomes. In the present work, confined to male germinal cells, with their XX-sex chromosomes it is difficult to distinguish the equal sized XX-sex chromosome pair in most cases. However, in few cases a relatively larger bivalent, as discussed above, has been observed in *Earias fabia* and *Sesamia inferens*. If the opinions of Bauer (1943), Suomalainen (1969), White (1973) and Ennis (1976) are to be taken as correct, we may consider the larger bivalent in such species as the XX-sex chromosome pair. Further in some of the species, with all small chromosomes of equal size, a peculiar type of lagging behaviour at anaphase-I has been shown by one chromosome pair as in *Acontia intersepta, Anomis sabulifera, Cosmophila erosa, Heliothis armigera, Plusia signata* and *Sesamia inferens*. This pair may be suspected as the XX-sex chromosome pair, but the point requires further investigation.

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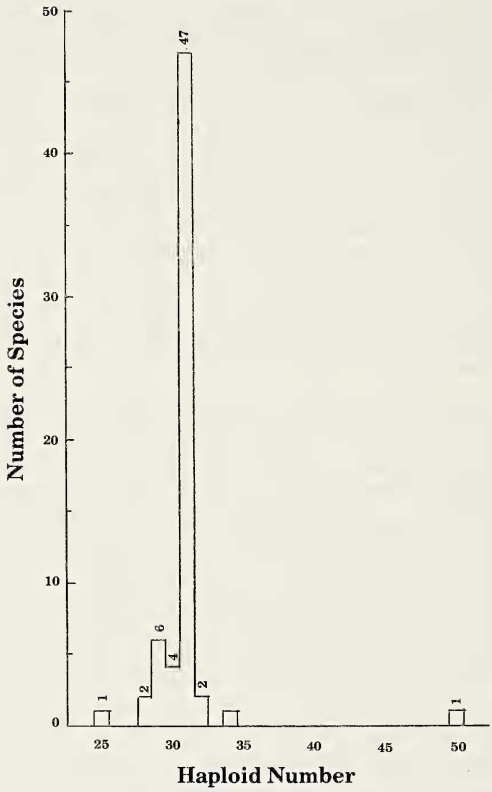

Fig. 34. Histogram showing haploid chromosome number in Family Noctuidae.

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